

Listing of the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1 - 175. (Canceled)

176. (Currently amended) A composition comprising mouse or primate embryonic stem cells and a serum-free cell culture medium capable of preventing differentiation of the mouse or primate embryonic stem cells during expansion of the embryonic stem cells, wherein the serum-free cell culture medium comprises a lipid-rich serum albumin.

177. (Previously presented) The composition of claim 176,
wherein the composition is capable of being stored indefinitely at less than or equal to about -135°C , and

wherein embryonic stem cells of the composition can be cultivated after storing the composition at less than or equal to about -135°C .

178. (Currently amended) The composition of claim 176, wherein the embryonic stem cells are obtained from a ~~human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, bird, reptile, fish, or amphibian.~~

179. (Currently amended) The composition of claim ~~176~~178, wherein the embryonic stem cells are obtained from ~~an human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, or bird.~~

180. (Currently amended) The composition of claim ~~176~~179, wherein the albumin is bovine albumin ~~embryonic stem cells are obtained from a mouse, cow, goat, or sheep.~~

181. (Currently amended) The composition of claim ~~176~~180, wherein the embryonic stem cells are obtained from a mouse.

182. (Currently amended) The composition of claim ~~176~~179, wherein the embryonic stem cells are obtained from a human.

183. (Previously presented) The composition of claim 176, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

184. (Previously presented) The composition of claim 176, wherein the serum-free cell culture medium comprises leukemia inhibiting factor.

185. (Previously presented) The composition of claim 176, wherein the serum-free cell culture medium comprises steel factor.

186. (Previously presented) The composition of claim 176, wherein the serum-free cell culture medium comprises ciliary neurotrophic factor.

187. (Previously presented) The composition of claim 176, wherein the serum-free cell culture medium comprises oncostatin M.

188. (Currently amended) The composition of claim 176,
wherein the serum-free cell culture medium comprises a basal cell culture medium,
wherein the basal cell culture medium comprises a serum-free supplement, and
wherein the serum-free supplement comprises ~~an albumin, an albumin substitute,~~ an
amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an
insulin substitute, a collagen precursor, or a trace element.

189. (Previously presented) The composition of claim 188,
wherein the composition is capable of being stored indefinitely at less than or equal
to about -135°C , and

wherein embryonic stem cells of the composition can be cultivated after storing the
composition at less than or equal to about -135°C .

190. (Currently amended) The composition of claim 188, wherein the embryonic
stem cells are obtained from ~~a human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig,~~
~~cow, swine, dog, horse, cat, goat, sheep, bird, reptile, fish, or amphibian.~~

191. (Currently amended) The composition of claim ~~188~~190, wherein the embryonic stem cells are obtained from ~~an human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, or bird.~~

192. (Currently amended) The composition of claim ~~188~~191, wherein the serum-free cell culture medium comprises an amino acid, a vitamin, a transferrin, an antioxidant, an insulin, a collagen precursor, and a trace element ~~embryonic stem cells are obtained from a mouse, cow, goat, or sheep.~~

193. (Currently amended) The composition of claim ~~188~~192 wherein the embryonic stem cells are obtained from a mouse.

194. (Currently amended) The composition of claim ~~188~~191, wherein the embryonic stem cells are obtained from a human.

195. (Previously presented) The composition of claim 188, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

196. (Previously presented) The composition of claim 188, wherein the serum-free cell culture medium comprises leukemia inhibiting factor.

197. (Previously presented) The composition of claim 188, wherein the serum-free cell culture medium comprises steel factor.

198. (Previously presented) The composition of claim 188, wherein the serum-free cell culture medium comprises ciliary neurotrophic factor.

199. (Previously presented) The composition of claim 188, wherein the serum-free cell culture medium comprises oncostatin M.

200. (Previously presented) The composition of claim 188, wherein the albumin is bovine albumin.

201. (Previously presented) The composition of claim 188, wherein the albumin is human albumin.

202-203. (Canceled)

204. (Previously presented) The composition of claim 188, wherein the transferrin is bovine transferrin.

205. (Previously presented) The composition of claim 188, wherein the transferrin is human transferrin.

206. (Currently amended) The composition of claim ~~188~~205, wherein the transferrin is iron-saturated.

207. (Previously presented) The composition of claim 188, wherein the insulin is bovine insulin.

208. (Previously presented) The composition of claim 188, wherein the insulin is human insulin.

209. (Previously presented) The composition of claim 188, wherein the insulin is recombinant insulin.

210. (Currently amended) A composition comprising mouse or primate embryonic stem cells and a serum-free cell culture medium,

wherein the serum-free cell culture medium is obtained by combining a basal cell culture medium with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an albumin substitute,~~ and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

211. (Previously presented) The composition of claim 210,
wherein the composition is capable of being stored indefinitely at less than or equal to about -135°C , and

wherein embryonic stem cells of the composition can be cultivated after storing the composition at less than or equal to about -135°C .

212. (Currently amended) The composition of claim 210, wherein the embryonic stem cells are obtained from ~~a human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, bird, reptile, fish, or amphibian.~~

213. (Currently amended) The composition of claim ~~210~~212, wherein the embryonic stem cells are obtained from ~~an human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, or bird.~~

214. (Currently amended) The composition of claim ~~210~~213, wherein the serum-free cell culture medium comprises an amino acid, a vitamin, a transferrin, an antioxidant, an insulin, a collagen precursor, and a trace element ~~embryonic stem cells are obtained from a mouse, cow, goat, or sheep.~~

215. (Currently amended) The composition of claim 210~~214~~, wherein the embryonic stem cells are obtained from a mouse.

216. (Currently amended) The composition of claim 210~~213~~ wherein the embryonic stem cells are obtained from a human.

217. (Previously presented) The composition of claim 210, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

218. (Previously presented) The composition of claim 210, wherein the serum-free cell culture medium comprises leukemia inhibiting factor.

219. (Previously presented) The composition of claim 210, wherein the serum-free cell culture medium comprises steel factor.

220. (Previously presented) The composition of claim 210, wherein the serum-free cell culture medium comprises ciliary neurotrophic factor.

221. (Previously presented) The composition of claim 210, wherein the serum-free cell culture medium comprises oncostatin M.

222. (Previously presented) The composition of claim 210, wherein the albumin is bovine albumin.

223. (Previously presented) The composition of claim 210, wherein the albumin is human albumin.

224-225. (Canceled)

226. (Previously presented) The composition of claim 210, wherein the transferrin is bovine transferrin.

227. (Previously presented) The composition of claim 210, wherein the transferrin is human transferrin.

228. (Currently amended) The composition of claim 210~~227~~, wherein the transferrin is iron-saturated.

229. (Previously presented) The composition of claim 210, wherein the insulin is bovine insulin.

230. (Previously presented) The composition of claim 210, wherein the insulin is human insulin.

231. (Previously presented) The composition of claim 210, wherein the insulin is recombinant insulin.

232. (Currently amended) A product of manufacture comprising a container means,

wherein the container means contains mouse or primate embryonic stem cells and a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an albumin substitute, and at least one component selected from the group consisting of an~~ amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein a basal cell culture medium supplemented with the serum-free supplement is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

233. (Previously presented) The product of manufacture of claim 232 in a frozen state.

234. (Previously presented) The product of manufacture of claim 232 not containing leukemia inhibitory factor.

235. (Previously presented) The product of manufacture of claim 232 containing leukemia inhibiting factor.

236. (Previously presented) The product of manufacture of claim 232 containing steel factor.

237. (Previously presented) The product of manufacture of claim 232 containing ciliary neurotrophic factor.

238. (Previously presented) The product of manufacture of claim 232 containing oncostatin M.

239. (Previously presented) The product of manufacture of claim 232, wherein the albumin is bovine albumin.

240. (Previously presented) The product of manufacture of claim 232, wherein the albumin is human albumin.

241. (Currently amended) The product of manufacture of claim ~~232~~240, wherein the serum-free cell culture medium comprises an amino acid, a vitamin, a transferrin, an antioxidant, an insulin, a collagen precursor, and a trace element~~-albumin is a lipid-rich.~~

242. (Canceled)

243. (Previously presented) The product of manufacture of claim 232, wherein the transferrin is bovine transferrin.

244. (Previously presented) The product of manufacture of claim 232, wherein the transferrin is human transferrin.

245. (Currently amended) The product of manufacture of claim ~~232~~244, wherein the transferrin is iron-saturated.

246. (Previously presented) The product of manufacture of claim 232, wherein the insulin is bovine insulin.

247. (Previously presented) The product of manufacture of claim 232, wherein the insulin is human insulin.

248. (Previously presented) The product of manufacture of claim 232, wherein the insulin is recombinant insulin.

249. (Currently amended) A product of manufacture comprising a container means,

wherein the container means contains mouse or primate embryonic stem cells and a serum-free cell culture medium,

wherein the serum-free cell culture medium comprises a basal cell culture medium,

wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an albumin substitute,~~ and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

250. (Currently amended) A product of manufacture comprising a container means,

wherein the container means contains mouse or primate embryonic stem cells and a serum-free cell culture medium,

wherein the serum-free cell culture medium is obtained by combining a basal cell culture medium with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an albumin substitute,~~ and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

251. (Currently amended) A product of manufacture comprising a first container means and a second container means,

wherein the first container means contains a serum-free supplement,

wherein the second container means contains mouse or primate embryonic stem cells,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an albumin substitute,~~ and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein a basal cell culture medium supplemented with the serum-free supplement is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

252. (Previously presented) The product of manufacture of claim 251 further comprising a third container means containing a basal medium.

253. (Currently amended) A product of manufacture comprising a first container means and a second container means,

wherein the first container means contains a serum-free cell culture medium,

wherein the second container means contains mouse or primate embryonic stem cells,

wherein the serum-free cell culture medium comprises a basal cell culture medium,

wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an albumin substitute,~~ and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, or ~~and~~ a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

254. (Currently amended) A product of manufacture comprising a first container means and a second container means,

wherein the first container means contains a serum-free cell culture medium,

wherein the second container means contains mouse or primate embryonic stem cells,

wherein the serum-free cell culture medium is obtained by combining a basal cell culture medium with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an albumin substitute,~~ and at least one component selected from the group consisting of an

amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

255. (Currently amended) A method for expanding mouse or primate embryonic stem cells comprising contacting the embryonic stem cells with a serum-free cell culture medium capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells, wherein the serum-free cell culture medium comprises a lipid-rich serum albumin.

256. (Previously presented) The method of claim 255 further comprising seeding the embryonic stem cells upon a layer of feeder cells.

257. (Previously presented) The method of claim 256, wherein the feeder cells are primary embryonic fibroblasts, inactivated feeder cells, or STO cells.

258. (Previously presented) The method of claim 255, wherein the serum-free cell culture medium does not contain leukemia inhibitory factor.

259. (Previously presented) The method of claim 255, wherein the serum-free cell culture medium comprises leukemia inhibiting factor.

260. (Previously presented) The method of claim 255, wherein the serum-free cell culture medium comprises steel factor.

261. (Previously presented) The method of claim 255, wherein the serum-free cell culture medium comprises ciliary neurotrophic factor.

262. (Previously presented) The method of claim 255, wherein the serum-free cell culture medium comprises oncostatin M.

263. (Currently amended) A method for expanding mouse or primate embryonic stem cells in a serum-free cell culture medium comprising

- (a) contacting the embryonic stem cells with a serum-free cell culture medium, and
- (b) expanding the embryonic stem cells,

wherein the serum-free cell culture medium comprises a basal cell culture medium, wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an~~ albumin substitute, and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

264. (Previously presented) The method of claim 263, wherein the albumin is bovine albumin.

265. (Previously presented) The method of claim 263, wherein the albumin is human albumin.

266-267. (Canceled)

268. (Previously presented) The method of claim 263, wherein the transferrin is bovine transferrin.

269. (Previously presented) The method of claim 263, wherein the transferrin is human transferrin.

270. (Currently amended) The method of claim ~~263~~269, wherein the transferrin is iron-saturated.

271. (Previously presented) The method of claim 263, wherein the insulin is bovine insulin.

272. (Previously presented) The method of claim 263, wherein the insulin is human insulin.

273. (Previously presented) The method of claim 263, wherein the insulin is recombinant insulin.

274. (Currently amended) A method for preventing the differentiation of mouse or primate embryonic stem cells in a serum-free cell culture medium comprising

(a) contacting the embryonic stem cells with a serum-free cell culture medium, and

(b) expanding the embryonic stem cells,

wherein the serum-free cell culture medium comprises a basal cell culture medium,

wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an albumin substitute,~~ and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

275. (Currently amended) A method for preventing the differentiation of mouse or primate embryonic stem cells in a serum-free cell culture medium comprising

(a) contacting the embryonic stem cells with a serum-free cell culture medium, and

(b) expanding the embryonic stem cells,

wherein the serum-free cell culture medium is obtained by combining a basal cell culture medium with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an albumin substitute,~~ and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

276. (Currently amended) A method for causing mouse or primate embryonic stem cells to differentiate in a serum-free cell culture medium comprising

(a) contacting the embryonic stem cells with a serum-free cell culture medium,

(b) expanding the embryonic stem cells, and

(c) adding a differentiation factor or changing culturing conditions thereby causing the embryonic stem cells to differentiate,

wherein the serum-free cell culture medium comprises a basal cell culture medium,

wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an albumin substitute,~~ and at least one component selected from the group consisting of an

amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

277. (Currently amended) A method for causing mouse or primate embryonic stem cells to differentiate in a serum-free cell culture medium comprising

(a) contacting the embryonic stem cells with a serum-free cell culture medium,

(b) expanding the embryonic stem cells, and

(c) adding a differentiation factor or changing culturing conditions thereby causing the embryonic stem cells to differentiate,

wherein the serum-free cell culture medium is obtained by combining a basal cell culture medium with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, ~~an albumin substitute,~~ and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

278. (Currently amended) A method for obtaining mouse or primate embryonic stem cells in a serum-free cell culture medium comprising

(a) isolating the embryonic stem cells from blastocysts, and
(b) expanding the embryonic stem cells in a serum-free cell culture medium,
wherein the serum-free cell culture medium comprises a basal cell culture medium,
wherein the basal cell culture medium is supplemented with a serum-free
supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin,~~an~~
~~albumin substitute,~~ and at least one component selected from the group consisting of an
amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an
insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation
of the embryonic stem cells during expansion of the embryonic stem cells.

279. (Currently amended) A method for obtaining mouse or primate embryonic
stem cells in a serum-free cell culture medium comprising

(a) isolating the embryonic stem cells from blastocysts, and
(b) expanding the embryonic stem cells in a serum-free cell culture medium,
wherein the serum-free cell culture medium is obtained by combining a basal cell
culture medium with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin,~~an~~
~~albumin substitute,~~ and at least one component selected from the group consisting of an
amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an
insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

280. (Currently amended) A method for producing a recombinant protein in mouse or primate embryonic stem cells in a serum-free cell culture medium comprising

(a) obtaining a recombinant mouse or primate embryonic stem cell containing a nucleic acid molecule encoding a recombinant protein,

(b) contacting the recombinant embryonic stem cell with a serum-free cell culture medium,

(c) expanding the recombinant embryonic stem cell in the serum-free cell culture medium to form a population of recombinant embryonic stem cells, and

(d) isolating the recombinant protein from the population of recombinant embryonic stem cells or from the serum-free cell culture medium of (c),

wherein the basal cell culture medium is supplemented with a serum-free supplement,

wherein the serum-free supplement comprises ~~an~~ a lipid-rich serum albumin, an albumin substitute, and at least one component selected from the group consisting of an amino acid, a vitamin, a transferrin, a transferrin substitute, an antioxidant, an insulin, an insulin substitute, a collagen precursor, ~~or~~ and a trace element, and

wherein the serum-free cell culture medium is capable of preventing differentiation of the embryonic stem cells during expansion of the embryonic stem cells.

281. (Currently amended) The method of claim 280 ~~282~~ wherein the recombinant protein is isolated from the population of recombinant embryonic stem cells.

282. (Currently amended) The method of claim 280, ~~282~~, wherein the recombinant protein is isolated from the serum-free cell culture medium of (c).